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EXAMINER
NGUYEN, QUANG

ART UNIT	PAPER NUMBER
1636	10

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/617,116	AGHI ET AL.	
	Examiner	Art Unit	
	Quang Nguyen	1636	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondenc addr ss --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *08 March 2002* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-3 and 5-13* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-3 and 5-13* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Claims 1-3 and 5-13 are pending in the present application.

Election/Restrictions

Applicants' election with traverse the invention of Group I (claims 1-3 and 5-13) in Paper No. 9 is acknowledged.

Applicants argued that Examiner has made an improper restriction requirement by placing claims 1-3, 5-6, 11 and 13 in all three restriction groups, and that Examiner has attempted to create restriction groups by reading into certain claims limitations regarding to the identity of the vector for gene delivery that are not present in the claims. Applicants' arguments are found unpersuasive for the following reasons. Firstly, Applicants are improperly incorporating multiple inventions in the dependent claim 11. Secondly, the multiple inventions encompassed within a vector for gene delivery include: (a) a non-viral or viral vector; (b) an endothelial and (c) a macrophage, which lack unity of invention. There is no common substantial structure feature among a viral or non-viral vector with an endothelial cell or with a macrophage or between an endothelial cell and a macrophage as a vector for gene delivery. As such, the methods utilizing these vectors for gene delivery comprise materially distinct processing steps, starting materials and require different technical considerations for delivering into neoplastic cells a nucleotide molecule encoding FPGS to attain the desired end-results (e.g., killing neoplastic cells with the presence of an antifolate drug), and therefore appear constitute patentably distinct inventions. Thirdly, Applicants are entitled for a

single patentably distinct invention to be examined per application. Examiner also noted that Applicants do not provide reasons or explanation why the aforementioned vectors of gene delivery and their associated methods do not constitute patentably distinct inventions. Accordingly, restriction for examination purposes as indicated is proper.

With respect to the linking claims, Applicants argued that "if claims 1-3, 5-6, 11 and 13 are indeed linking claims, as asserted by the Examiner, then under MPEP § 814, it is improper to associate them with any of the restriction groups". Applicants' argument is unpersuasive because consistent with MPEP § 814, the linking claims have been grouped together with claims drawn to a single patentably distinct invention. It is noted that Applicants have improperly incorporated multiple inventions within a single independent claim for the reasons set forth in the preceding paragraph.

Applicants further argued that the Examiner has added limitations that are not included within many of the claims as currently written in an attempt to place the claims into different restriction groups. Again, Applicants' argument is found unpersuasive because Applicants have improperly incorporated multiple inventions within a single independent claim 11 for the reasons set forth above. It should be noted that Applicants are entitled for a single patentably distinct invention to be examined per application

Applicants' election with traverse for the following species: (a) prokaryotic vector for a non-viral vector, and (b) retrovirus for a viral vector is acknowledged. The reason for the traverse election of species is based on the ground that an improper group restriction requirement for the reasons set forth above. Applicants' arguments related to

an improper group restriction requirement are found unpersuasive for the reasons discussed above.

Accordingly, restriction for examination purposes as indicated in Paper No. 8 is proper. This is made FINAL.

Following is a new ground of rejection.

Written Description

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-2 and 5-13 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Vas-Cath Inc. v. Mahurkar, 19USPQ2d 1111 (Fed. Cir. 1991), clearly states that "applicant must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession *of the invention.* The invention is, for purposes of the 'written description' inquiry, *whatever is now claimed.*" Vas-Cath Inc. v. Mahurkar, 19USPQ2d at 1117. The specification does not "clearly allow persons of ordinary skill in the art to recognize that [he or she] invented what is claimed."Vas-Cath Inc. v. Mahurkar, 19USPQ2d at 1116.

Applicant's invention is drawn to a method for killing neoplastic cells comprising delivering into the neoplastic cells a vector for gene delivery, said vector comprising a nucleotide molecule encoding folylpolyglutamyl synthetase (FPGS), followed by treating the neoplastic cells containing said nucleotide molecule with an antifolate drug that results the killing of the neoplastic cells. The instant claims encompass the utilization of a nucleotide molecule encoding FPGS derived from any species (both mammalian and non-mammalian species). However, apart from the exemplification showing a human FPGS cDNA gene product that is capable of polyglutamating antifolate drugs (e.g., methotrexate, edatrexate) to enhance their cytotoxicity or to increase their therapeutic efficacy, the instant specification fails to teach a representative number of species of nucleotide sequences encoding FPGS derived from any organisms that are capable of meditating the same desired anti-neoplastic cell effects as encompassed within a broad scope of the instant claims. At the effective filing date of the present application, mouse and human FPGS genes are the only two mammalian FPGS genes that have been cloned and sequenced along with the bacterial FPGS genes from *E. Coli* and *Lactobacillus casei* (Garrow et al., Proc. Natl. Acad. Sci. 89:9151-9155, 1992; IDS; Spinella et al., Biochim. Biophys. Acta 7: 1305:11-14, 1996, abstract only). Furthermore, unlike mammalian FPGS, the bacterial enzymes can only metabolize folates to short polyglutamate derivatives and that they display a folate specificity quite distinct from the mammalian enzymes along with limited areas of homology between their sequences and the human FPGS sequence (Garrow et al., Proc. Natl. Acad. Sci. 89:9151-9155, 1992; IDS). The claimed invention as a whole is not adequately

described if the claims require essential or critical elements which are not adequately described in the specification, and which are not conventional in the art as of Applicants' filing date. Possession may be shown by actual reduction to practice, clear depiction of the invention in a detailed drawing, or by describing the invention with sufficient relevant identifying characteristics such that a person skilled in the art would recognize that the inventor had possession of the claimed invention. Pfaff v. Wells Electronics, Inc.; 48 USPQ2d 1641, 1646 (1998). The skilled artisan cannot fully envision the detailed structure of any nucleotide molecule encoding mammalian FPGS apart from those encoding mouse and human FPGS, or any nucleotide molecule encoding non-mammalian FPGS apart from those encoding *E. Coli* and *Lactobacillus casei*. FPGS, and therefore conception is not achieved until reduction to practice has occurred, regardless of the complexity or simplicity of the method. Adequate written description requires more than a mere statement that it is part of the invention and reference to a potential method of isolating it. See *Fiers v. Revel*, 25 USPQ2d 1601, 1606 (Fed. Cir. 1993) and *Amgen Inc. v. Chugai Pharmaceutical Co. Ltd.*, 18 USPQ2d 1016 (Fed. Cir. 1991). One cannot describe what one has not conceived. See *Fiddes v. Baird*, 30 USPQ2d 1481, 1483.

Applicant is reminded that *Vas-Cath* makes clear that the written description provision of 35 U.S.C. §112 is severable from its enablement provision (see page 1115).

Claim R j ctions - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> **The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.**

Claims 1-3 and 5-13 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for:

(1) A method for killing neoplastic cells *in vitro*, said method comprising: (a) transforming or transfecting said neoplastic cells with a vector comprising a DNA sequence encoding human, mouse, *E.Coli* or *Lactobacillus casei* folylpolyglutamate synthetase (FPGS) operably linked to a promoter, wherein the FPGS is expressed in the neoplastic cells; (b) treating the neoplastic cells of step (a) with an antifolate drug that is polyglutamated by the FPGS; whereby the neoplastic cells are killed and wherein said vector is a non-viral vector or a replication defective viral vector;

(2) A method for killing neoplastic cells *in vivo*, said method comprising: (a) direct innoculation of said neoplastic cells with a vector comprising a DNA sequence encoding human, mouse, *E.Coli* or *Lactobacillus casei* folylpolyglutamate synthetase (FPGS) operably linked to a promoter, wherein the FPGS is expressed in the neoplastic cells; (b) treating the neoplastic cells in step (a) with an antifolate drug that is polyglutamated by the FPGS; whereby the neoplastic cells are killed and wherein said vector is a non-viral vector or a replication defective viral vector;

does not reasonably provide enablement for other embodiments of the claims. The specification does not enable any person skilled in the art to which it pertains, or

with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

The factors to be considered in the determination of an enabling disclosure have been summarized as the quantity of experimentation necessary, the amount of direction or guidance presented, the state of the prior art, the relative skill of those in the art, the predictability or unpredictability of the art and the breadth of the claims. *Ex parte Forman*, (230 USPQ 546 (Bd Pat. Appl & Unt, 1986); *In re Wands*, 858 F.2d 731, 8 USPQ 2d 1400 (Fed. Cir. 1988)).

The claims are drawn to a method for killing neoplastic cells, said method comprising: (a) delivering into said neoplastic cells with a vector for gene delivery, said vector comprising a nucleotide molecule encoding FPGS, wherein said nucleotide molecule directs the production of said FPGS in said neoplastic cells containing said nucleotide molecule; (b) treating said neoplastic cells containing said nucleotide molecule with an antifolate drug; and killing said neoplastic cells containing said nucleotide molecule; the same method wherein said FPGS is a mammalian FPGS, preferably a human FPGS, or wherein said antifolate drug is methotrexate, edatrexate, aminopterin or a thymidylate synthetase inhibitor or wherein said vector is a viral vector or a non-viral vector.

The specification teaches by exemplification showing that in comparison with parental 9L rat gliosarcoma cells, 9L/FPGS cells stably transfected with a plasmid vector comprising a human FPGS cDNA are more sensitive to the antifolate drugs such as methotrexate, edatrexate and aminopterin in cell cultures in 4-hour pulses of

antifolates. Additionally, the specification teaches subcutaneous implanted 9L/FPGS tumors in nude mice also respond well to the treatments of methotrexate and edatrexate. Applicants further disclose a bystander killing effect of non-transfected tumor cells was observed in both *in vitro* and *in vivo* resulting from the release of antifolates by transfected tumor cells after the removal of extracellular drugs. The evidence has been noted and considered, however the evidence is not reasonably extrapolated to the instant broadly claimed invention for the following reasons.

The instant claimed invention encompasses both *in vitro* and *in vivo* methods of killing neoplastic cells utilizing a vector for gene delivery comprising a nucleotide molecule encoding FPGS together with an antifolate drug that is activated by the FPGS gene product to effect the killing of the neoplastic cells. The broad claims encompass the utilization of any nucleotide molecule encoding FPGS derived from any mammalian and/or non-mammalian sources to enhance the cytotoxic or therapeutic effects of an antifolate drug to kill neoplastic cells. The present specification is not enabled for the full scope of the method as claimed for the reasons set forth in the Written Description section above. Given the lack of guidance provided by the instant specification regarding to the availability of a nucleotide sequence encoding any FPGS other than those encoding human, mouse, *E.Coli* or *Lactobacillus* casei FPGS, , it would have required undue experimentation for one skilled in the art to make and use the full scope of the methods as claimed. With respect to the breadth of the instant claims regarding to the utilization of any nucleotide molecule encoding FPGS, Applicants' attention is directed to the decision in *In re Shokal*, 113 USPQ 283 (CCPA 1957) wherein is stated:

> It appears to be well settled that a single species can rarely, if ever, afford sufficient support for a generic claim. In re Soll, 25 C.C.P.A. (Patents) 1309, 97 F.2d 623, 38 USPQ 189; In re Wahlforss et al., 28 C.C.P.A. (Patents) 867, 117 F.2d 270, 48 USPQ 397. The decisions do not however fix any definite number of species which will establish completion of a generic invention and it seems evident therefrom that such number will vary, depending on the circumstances of particular cases. Thus, in the case of small genus such as the halogens, consisting of four species, a reduction to practice of three, or perhaps even two, might serve to complete the generic invention, while in the case of a genus comprising hundreds of species, a considerably larger number of reductions to practice would probably be necessary.

With respect to the *in vivo* aspect of the claimed methods, the nature of the claimed invention falls within the art of *in vivo* gene therapy that remains to be unpredictable for obtaining therapeutic effects at the effective filing date of the present application. Dang et al. (Clin. Cancer Res. 5:471-474, 1999) noted that further advancement in all fields such as gene delivery, gene expression and host immune manipulation is needed to make gene therapy a reality. Dang et al. also pointed out several factors limiting an effective gene therapy, including sub-optimal vectors, the lack of a stable *in vivo* transgene expression, the adverse host immunological responses to the delivered vectors and most importantly an efficient gene delivery to target tissues or cells (last paragraph, col. 2, page 474). The broad claims encompass any route of delivering a vector of gene delivery comprising a nucleotide molecule encoding FPGS into neoplastic cells *in vivo*. However, vector targeting *in vivo* to desired cells or tissues, for this instance neoplastic cells, continues to be unpredictable and inefficient. This is supported by numerous teachings in the art. As examples, Miller & Vile (FASEB 9:190-199, 1995) reviewed the types of vectors available for *in vivo* gene therapy, and concluded that "for the long-term success as well as the widespread applicability of human gene therapy, there will have to be advances ... Targeting strategies outlined in this review, which are currently only at the experimental level, will have to be translated

into components of safe and highly efficient delivery systems" (page 198, column 1). Deonarain (Exp. Opin. Ther. Patents 8:53-69, 1998) indicated that one of the main obstacles hampering a successful gene therapy is the "ability to target a gene to a significant population of cells and express it at adequate levels for a long enough period of time." (page 53, first paragraph). Deonarain also reviewed new techniques under experimentation in the art that show promises. One of which is the ligand-targeted receptor-mediated vector approach with a relatively higher level of tissue specificity than viruses can offer. However, this approach to gene therapy is much less efficient than viral gene delivery (column 1, last paragraph, page 65). Verma & Somia (Nature 389:239-242,1997) reviewed various vectors known in the art for use in gene therapy, and the problems that are associated with each. They indicated clearly that resolution to vector targeting *in vivo* had not been achieved in the art (see the entire article). Verma & Somia also discussed the role of the host immune system in inhibiting an efficient targeting of viral vectors to desired cells and tissues (see page 239, and second and third columns of page 242). Verma & Somia also indicated that appropriate enhancer-promoter sequences can improve expression, but that the "search for such combinations is a case of trial and error for a given cell type." (page 240, sentence bridging columns 2 and 3). The instant specification fails to teach one of skilled in the art how to overcome the unpredictability for *in vivo* vector targeting, such that an efficient transfer and expression of a nucleotide molecule encoding FPGS could be achieved in neoplastic cells *in vivo* through any route of delivery such that upon treatment with an antifolate drug, the drug is activated by the FPGS gene product to

effect the killing of said neoplastic cells. The exemplification demonstrating the sensitivity of 9L/FPGS rat gliosarcoma cells stably transfected *in vitro* with a plasmid vector comprising a human FPGS cDNA to treatments of antifolate drugs such as methotrexate, edatrexate is not deemed to be sufficient guidance for one skilled in the art for overcoming the unpredictability of *in vivo* vector targeting to attain therapeutic effects. As such, with the lack of sufficient guidance provided by the present specification, it would have required undue experimentation for a skilled artisan to make and use the instant broadly claimed invention.

Regarding to the *in vivo* aspect of the claimed methods, the instant claims encompass the use of replication competent viral vectors. Neither the instant specification nor the prior art at the effective filing date of the present application teaches the use of replication competent viral vectors such as retrovirus, adenovirus or lentivirus (HIV-1 and HIV-2) for achieving therapeutic results via gene therapy; particularly by any route of administration including a systemic delivery of recombinant replication competent viral vectors. It is unclear whether the treated individual having neoplastic cells succumbs to the cytotoxic effects of replication competent viral vectors prior to any therapeutic effects contemplated by Applicants could be attained. Furthermore, neoplastic cells infected with replication competent viruses can be lysed or killed by replication competent recombinant viruses prior to any effective accumulation of polyglutamated antifolates could be attained in the infected neoplastic cells to mediate the killing of neoplastic cells as contemplated by Applicants. With the lack of guidance provided by the specification regarding to this embodiment of the claims, it

would have required undue experimentation for a skilled artisan to make and use the full scope of the methods as claimed.

With respect to claim 11, specifically encompassing the use of mammalian artificial chromosome as a non-viral gene delivery of FPGS gene for killing neoplastic cells. However, the instant specification fails to provide any specific teachings regarding to the making or using of any mammalian artificial chromosome for killing a neoplastic cell in a method as claimed. Furthermore, with respect to the issue of mammalian artificial chromosome, Calos (TIG 12:463-467, 1996; PTO-1449, AT2) noted that "a vector of this size is far beyond the size of vectors in current use for gene therapy and poses problems of major dimensions, particularly for the manufacture and delivery of vector DNA. Therefore, while construction of artificial chromosome vectors has not yet been realized, once it is, a series of challenging technical barriers will have to be surmounted before such molecules could reasonably be used as gene therapy vectors" (page 464, col. 2, last paragraph). Therefore, with the lack of guidance provided by the instant specification, particularly in the absence of any *in vivo* example demonstrating an effective use of an artificial chromosome vector comprising a nucleotide sequence encoding FPGS for killing neoplastic cells *in vivo*, it would have required undue experimentation for a skilled artisan to make and use this particularl embodiment of the presently claimed invention.

Accordingly, due to the lack of guidance provided by the specification regarding to the issues set forth above, the unpredictability of the gene therapy art, and the

breadth of the claims, it would have required undue experimentation for one skilled in the art to make and use the instant broadly claimed invention.

Response to Arguments

Applicants' arguments related to the above rejection in the Amendment filed on November 29, 2001 in Paper No. 7 (pages 5-16) have been fully considered.

With respect to the issue of the administering route, Applicants mainly argued that "the Examiner has not established that the *in vivo* delivery of genetic vectors by methods other than direct injection is of such low efficiency so as to be regarded as impracticable". Applicants further argued that at the filing date of the present application, the scientific literature was replete with examples of successful *in vivo* genetic vector delivery using methods other than direct injection as evidenced by the teachings of Deonarain (page 59, left column), Lan et al/ and Nakanishi. Moreover, Applicants argued that the claims do not require the vector for gene delivery to be delivered into neoplastic cells with any particular minimal level of efficiency, and that successful application of the invention does not require high levels of efficiency of genetic vector delivery. Applicants' arguments are found unpersuasive for the following reasons.

Firstly, the referred paragraph in the reference of Deonarain merely indicates that liver can take up 85% of the injected DNA molecule via the vascular system. It is also noted that Deonarain teaches that even in the absence of specific targeting, many molecules can be delivered to the liver since one-fifth of the cardiac output flows

through the liver per minute (page 59, bottom of first full paragraph). Deonarain does not teach that the injected DNA molecule could be delivered efficiently to neoplastic cells occurring elsewhere in a patient, for examples in the brain, skin, breast or other organ or tissues to yield the therapeutic effects contemplated by Applicants, particularly the majority of the delivered vector would be sequestered in the liver. It is also noted that the instant claimed invention is not drawn to killing neoplastic cells in a liver.

Secondly, with respect to the cited reference of Lan et al., it is noted that the recombinant adenoviral vector was intraperitoneally injected into nude mice bearing tumor xenografts. The nude mice are known to lack functional immune responses, and therefore they do not represent a typical *in vivo* situation of a patient having neoplastic cells. In a host with a competent immune system, it is well known that the adverse host immune response against recombinant adenoviral vectors would limit an effective amount of transgene to be delivered into desired target cells or tissues to attain contemplated therapeutic outcomes as evidenced by the teachings of Verma et al. discussed above.

Thirdly, with respect to the reference of Nakanishi, the mere statement that "*In vivo* gene transfer is an approach to transfect tissue cells *in situ* by introducing gene transfer vectors through direct injection, through perfusion with catheters, or through an intravenous injection" does not indicate that the unpredictability and inefficient vector targeting *in vivo* to desired cells or tissues known in the art has been overcome, particularly for attaining therapeutic results. Even several years after the publication of the review of Nakanishi, Dang et al. still pointed out the lack of an efficient gene delivery

to target tissues or cells still limits the effectiveness of gene therapy (last paragraph, col. 2, page 474). Verma & Somia also indicated clearly that resolution to vector targeting *in vivo* had not been achieved in the art in 1997 (see the entire article), let alone at the time Nakanishi published his review article.

In light of the state of the gene therapy art at the filing date of the present application, and the absence of sufficient guidance provided by the present application particularly without any *in vivo* example demonstrating that obstacles associated with *in vivo* vector targeting has been overcome, it would have required undue experimentation for a skilled artisan to make and use the methods as claimed.

With respect to the issue of replication competent viral vectors, Applicants argued that "the Examiner has not provided any objective evidence in support of his apparent conclusion that the use of replication competent viral vectors would therefore interfere with the practice of the claimed invention", and that the presence of inoperative embodiment within the scope of a claim does not render a claim non-enabled. Applicants' arguments are found unpersuasive because Examiner clearly noted that it would have required undue experimentation for a skilled artisan to practice the methods as claimed since neither the instant specification nor the prior art at the effective filing date of the present application teaches the use of replication competent viral vectors such as retrovirus, adenovirus or lentivirus (HIV-1 and HIV-2) for achieving therapeutic results via gene therapy; particularly by any route of administration including a systemic delivery of recombinant replication competent viral vectors. Additionally, neoplastic cells infected with replication competent viruses can be lysed or killed by replication

competent recombinant viruses prior to any effective accumulation of polyglutamated antifolates could be attained in the infected neoplastic cells to mediate the killing of neoplastic cells as contemplated by Applicants. Applicants have not provided any factual evidence to indicate otherwise. Applicants should be further noted that the scope of the claims must bear a reasonable correlation to scope of enblement provided by the specification as set forth in *In re Fisher*, 166 USPQ 18 (CCPA 1970).

With respect to the issue of mammalian artificial chromosome, Applicants again argued that "the Examiner has provided no evidence or arguments to suggest that the reasonable practice of the claimed invention would be substantially impeded due to the presence of mammalian artificial chromosomes within the scope of the claims. Nor has the Examiner set forth any indication that it would require undue experimentation to distinguish the operative from the supposed inoperative embodiments of the claimed invention". Applicants' arguments are found unpersuasive because the instant specification fails to provide any specific teachings regarding to the making or using of any mammalian artificial chromosome for killing a neoplastic cell in a method as claimed, particularly in light of the teachings of Calos (TIG 12:463-467, 1996; PTO-1449, AT2) who noted that "a vector of this size is far beyond the size of vectors in current use for gene therapy and poses problems of major dimensions, particularly for the manufacture and delivery of vector DNA. Therefore, while construction of artificial chromosome vectors has not yet been realized, once it is, a series of challenging technical barriers will have to be surmounted before such molecules could reasonably be used as gene therapy vectors" (page 464, col. 2, last paragraph). Even assuming

that artificial chromosome vectors can be constructed, there is no factual evidence even in the year 2002 that they can be efficiently transfected or transformed neoplastic cells *in vivo* by any route of delivery to attain the desired therapeutic effects contemplated by Applicants (e.g., killing neoplastic cells). In the absence of sufficient guidance provided by the present application, it would have required undue experimentation for a skilled artisan to make and use the methods as claimed. Applicants are reminded once again that the scope of the claims must bear a reasonable correlation to scope of enblement provided by the specification as set forth in *In re Fisher*, 166 USPQ 18 (CCPA 1970).

Accordingly, claims 1-3 and 5-13 are rejected under 35 U.S.C. 112, first paragraph, for the reasons set forth above.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-3 and 5-11 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

In amended claim 1 and its dependent claims, the phrase "said nucleotide molecule directs the production of said FPGS" in step (a) is unclear. How can a nucleotide molecule encoding folylpolyglutamyl synthetase (FPGS) also direct the production of FPGS? As an example, by simply providing a cDNA molecule encoding FPGS, would such a molecule direct the expression or production of FPGS? It should be noted that the function of an encoding nucleotide molecule is distinct from that of a

promoter which directs the expression and subsequent production of a DNA sequence encoding FPGS operably linked to said promoter. Clarification is requested.

Claim 6 recites the limitation "said FPGS gene" and "said chemotherapeutic agent" in lines 1-2 of the claim. There is insufficient antecedent basis for this limitation in the claim. There is no recitation of FPGS gene or chemotherapeutic agent in claim 1 from which claim 6 is dependent upon.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1-3, 5-6 and 10-12 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kim et al. (J. Biol. Chem. 268:21680-21685, 1993; PTO-1449, AS) as

evidenced by Osborne et al. (J. Biol. Chem. 268: 21657-21664; 1993) and in view of Garrow et al. (Proc. Natl. Acad. Sci. 89:9151-9155, 1992; PTO-1449, AR) and Roy et al. (J. Biol. Chem. 272:6903-6908, 1997; IDS).

The claims are drawn to a method for killing neoplastic cells, said method comprising: (a) delivering into said neoplastic cells with a vector for gene delivery, said vector comprising a nucleotide molecule encoding FPGS, wherein said nucleotide molecule directs the production of said FPGS in said neoplastic cells containing said nucleotide molecule; (b) treating said neoplastic cells containing said nucleotide molecule with an antifolate drug; and killing said neoplastic cells containing said nucleotide molecule; the same method wherein said FPGS is a mammalian FPGS, preferably a human FPGS, or wherein said antifolate drug is methotrexate, edatrexate, aminopterin or a thymidylate synthetase inhibitor or wherein said vector is a non-viral vector.

With respect to the enabled scope of an *in vitro* method and the elected species, Kim et al. teaches that FPGS-deficient mutant Chinese hamster ovary (CHO AUXB1) cells expressing human folylpolyglutamate synthetase (FPGS) metabolize methotrexate (MTX) to polyglutamates characteristics of human cells (see Table I, page 21681), and that upon a short term exposure to MTX (4 h or 72 h), cells expressing higher levels of human FPGS are more sensitive to the cytotoxicity of MTX compared to cells expressing lower levels of human FPGS (see Table III, page 21682). The CHO cells expressing human FPGS have been obtained by the co-transfection of CHO AUXB1 cells with sheared bulk human DNA and pSV2-neo plasmid as evidenced by the

teachings of Osborne et al. from the same research group (see page 21658, col. 2, last full paragraph). Kim et al. noted that the ability of cells to metabolize MT to longer chain length derivatives enhances cytotoxicity when MTX is infused for a limited period and then removed, which mimics clinical usage, and that larger effects of FPGS activity levels on the cytotoxicity of antifolates that require polyglutamylation for effective inhibition of target enzymes were also observed (page 21683, col. 2, last paragraph). Kim et al. further teach that lowered FPGS activity may be a general mechanism by which human leukemia cells can become resistant to a wide range of antifolates (page 21684, col. 1, top 5 lines) and that decreased polyglutamylation as a mechanism for inherent MTX resistance for a number of sarcoma and squamous carcinoma cell lines even though FPGS levels appear normal (page 21684, col. 1, bottom of the second paragraph). Kim et al. do not specifically teach the transformation or transfection of neoplastic cells with a vector comprising a DNA sequence encoding FPGS, followed by a treatment of an antifolate drug to effect the killing of said neoplastic cells.

However, at the filing date of the present application Garrow et al. teach the cloning of a human cDNA sequence encoding for FPGS, as well as the expression of human FPGS in mammalian CHO AUXB1 cells with a plasmid vector pSVK-hFPGS (see Fig. 1 and page 9152, col. 2, first full paragraph). Roy et al. teach that tumor cell variants with acquired resistance to methotrexate and other folate analogues exhibit lower levels of FPGS activity when compared with parental cells and are cross-resistant to most classical folate analogues. Specifically, Roy et al. teach that L1210 tumor cells resistant to methotrexate have a decrease in the rate of FPGS mRNA transcript

formation, resulting in lower FPGS activity (page 6907, col. 2, first full paragraph), and that L1210 tumor cells resistant to edatrexate have constitutively down-regulated steady state levels of FPGS or FPGS activity compared with parental L1210 tumor cells and that FPGS mRNA from the variant cells was significantly less effective in mediating formation of the FPGS peptide product in a manner correlating with the FPGS activity or protein (see abstract).

Accordingly, at the time of the instant invention it would have been obvious and within the skills of an ordinary skilled artisan to modify the method disclosed by Kim et al. by transforming a non-viral vector (or a prokaryotic vector) comprising a DNA sequence encoding human FPGS into neoplastic or tumor cells resistant to methotrexate and other folate analogues *in vitro* to enhance the cytotoxic effects of the antifolate drugs into said neoplastic or tumor cells in light of the teachings of Garrow et al. and Roy et al. One of ordinary skilled in the art would have been motivated to carry out the above modification to investigate whether the exogenous supply of FPGS into tumor cells resistant to methotrexate or folate analogues would restore cytotoxic sensitivity of these cancer cells to methotrexate or folate analogues. One of ordinary skilled artisan would have a reasonable expectation of success for the modified method because lowered FPGS activity and decreased polyglutamylation of antifolates are thought to be general mechanisms by which cancer cells become resistant to a wide range of antifolates, and that Roy et al. clearly show that L1210 tumor cells resistant to methotrexate or edatrexate have lowered FPGS activity, coupled with the teachings of

Kim et al. demonstrating that FPGS-deficient mutant CHO AUXB1 cells expressing high levels of human FPGS become more sensitive to the cytotoxicity of methotrexate.

Therefore, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Claims 1, 7-9 and 13 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kim et al. (J. Biol. Chem. 268:21680-21685, 1993; PTO-1449, AS) as evidenced by Osborne et al. (J. Biol. Chem. 268: 21657-21664; 1993) and in view of Garrow et al. (Proc. Natl. Acad. Sci. 89:9151-9155, 1992; PTO-1449, AR) and Roy et al. (J. Biol. Chem. 272:6903-6908, 1997; IDS) as applied to claims 1-3, 5-6 and 10-12 above, and further in view of Nakanishi (Critical Reviews in Therapeutic Drug Carrier System 12:263-310; IDS).

The combined teachings of Kim et al., Osborne et al., Garrow et al. and Roy et al. have been discussed above. However, none of the references specifically teach the use of a recombinant retrovirus comprising a DNA sequence encoding FPGS for transfection into tumor cells resistant to methotrexate or folate analogues. At the effective filing date of the present application and several years after the teachings of Kim et al., there are various viral vectors available for delivering a transgene into cells *in vitro* or *in vivo*. Nakanishi reviewed various viral vectors, including retrovirus, adenovirus, herpes virus and others, available for gene transfer applications (see page 268-270). Nakanishi also teach that particle bombardment as well as direct injection of

DNA molecules among others as means of gene transfer into cells *in vivo* and in cell cultures (see pages 278-281).

Accordingly, it would have been obvious and within the scope of skill for an ordinary skilled artisan to use a recombinant retrovirus vector for delivering a DNA sequence encoding human FPGS into neoplastic or tumor cells resistant to methotrexate and other folate analogues *in vitro* in the modified method resulting from the combined teachings of Kim et al., Osborne et al., Garrow et al. and Roy et al. as discussed above, including means of direct injection into the tumor cells or by particle bombardment as taught by Nakanishi. One of ordinary skilled artisan would have been motivated to carry out this modification simply on the designer's choice of vectors and/or means of delivering an exogenous source of FPGS into tumor cells resistant to methotrexate and other folate analogues.

Therefore, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Claims 1-3 and 5-13 are rejected under 35 U.S.C. 103(a) as being unpatentable over Roy et al. (J. Biol. Chem. 272:6903-6908, 1997; IDS) in view of Kim et al. (J. Biol. Chem. 268:21680-21685, 1993; PTO-1449, AS), Garrow et al. (Proc. Natl. Acad. Sci. 89:9151-9155, 1992; PTO-1449, AR) and Roth et al. (J. Natl. Cancer Inst. 89:21-39, 1997).

With respect to the enabled scope of an *in vivo* method and the elected species, Roy teach that a major limitation for cancer therapy with classical folate analogues is

the acquired resistance of tumor cells to methotrexate and folate analogues (page 6903, col. 2, first full paragraph). Specifically, Roy et al. teach that *in vitro* L1210 tumor cells resistant to methotrexate have a decrease in the rate of FPGS mRNA transcript formation, resulting in lower FPGS activity (page 6907, col. 2, first full paragraph), and that L1210 tumor cells resistant to edatrexate have constitutively down-regulated steady state levels of FPGS or FPGS activity compared with parental L1210 tumor cells and that FPGS mRNA from the variant cells was significantly less effective in mediating formation of the FPGS peptide product in a manner correlating with the FPGS activity or protein (see abstract). Although Roy et al. noted that the modifications in FPGS activity in the L1210 tumor variants have broad significance in regard to the regulation of FPGS gene expression and for therapy with classical folate analogues (page 6903, col. 2, bottom of first full paragraph), Roy et al. do not specifically teach to deliver directly into neoplastic cells *in vivo* a vector comprising a nucleotide molecule encoding FPGS, then treated the neoplastic cells containing the nucleotide molecule with an antifolate drug (e.g., methotrexate or edatrexate) to kill the neoplastic cells.

However, at the effective filing date of the present application, Kim et al. teaches that FPGS-deficient mutant Chinese hamster ovary (CHO AUXB1) cells expressing human folylpolyglutamate synthetase (FPGS) metabolize methotrexate (MTX) to polyglutamates characteristics of human cells (see Table I, page 21681), and that upon a short term exposure to MTX (4 h or 72 h), cells expressing higher levels of human FPGS are more sensitive to the cytotoxicity of MTX compared to cells expressing lower levels of human FPGS (see Table III, page 21682). Kim et al. noted that the ability of

cells to metabolize MT to longer chain length derivatives enhances cytotoxicity when MTX is infused for a limited period and then removed, which mimics clinical usage, and that larger effects of FPGS activity levels on the cytotoxicity of antifolates that require polyglutamylation for effective inhibition of target enzymes were also observed (page 21683, col. 2, last paragraph). Kim et al. further teach that lowered FPGS activity may be a general mechanism by which human leukemia cells can become resistant to a wide range of antifolates (page 21684, col. 1, top 5 lines) and that decreased polyglutamylation as a mechanism for inherent MTX resistance for a number of sarcoma and squamous carcinoma cell lines even though FPGS levels appear normal (page 21684, col. 1, bottom of the second paragraph). Garrow et al. teach the cloning of a human cDNA sequence encoding for FPGS, as well as the expression of human FPGS into the same CHO AUXB1 cells using a plasmid vector pSVK-hFPGS (see Fig. 1 and page 9152, col. 2, first full paragraph). Roth et al. review various gene therapy approaches utilizing viral and non-viral vectors that have resulted in the regression of tumors *in vivo* (see the entire article, especially, the section of Drug-sensitivity genes on pages 22-23). With respect to clinical applications, Roth et al. further noted that the administration of viral vectors to patients is limited to intratumoral delivery since the available vectors have not been approved for systemic administration, and that immune responses against administered vectors limit repetitive administration (page 24, col. 1, last paragraph continues to top of col. 2).

Accordingly, at the time of the instant invention it would have been obvious and within the scope of skills for an ordinary skilled artisan to direct delivery of a non-viral or

viral vector comprising a DNA sequence encoding human FPGS into neoplastic cells *in vivo* that may acquire resistance to methotrexate and other classical folate analogues in order to restore their sensitivity to the cytotoxic effects of these antifolate drugs in light of the above teachings. One of ordinary skilled in the art would have been motivated to carry out the above modified method because Kim et al. clearly teach that lowered FPGS activity and decreased polyglutamylation of antifolates are thought to be general mechanisms by which cancer cells become resistant to a wide range of antifolates, and that FPGS-deficient mutant Chinese hamster ovary (CHO AUXB1) cells expressing high levels of human folylpolyglutamate synthetase (FPGS) are more sensitive to the cytotoxicity of MTX compared to cells expressing lower levels of human FPGS (see Table III, page 21682). Furthermore, Roy et al. clearly show that L1210 tumor cells resistant to methotrexate or edatrexate have lowered FPGS activity. Therefore, one of ordinary skilled artisan would have a reasonable expectation of success by providing an exogenous supply of human FPGS into neoplastic cells that may acquire resistant to classical antifolate drugs *in vivo*, in the form of a recombinant viral or non-viral vector to enhance their FPGS activity or protein in order to render or restore them more sensitive to the cytotoxicity of the antifolate drugs. Furthermore, with respect to the enabled gene therapy aspect of the modified method, it would have been within the scope of skill for an ordinary skilled artisan to practice it on the basis of the teachings of Roth et al. who show that certain gene-based therapies for cancer, including drug sensitization with genes for prodrug delivery similar in nature with the presently claimed invention, have

resulted in tumor regressions or killing cancer cells (see the entire article, particularly pages 21-24).

Therefore, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Response to Arguments

Applicants' arguments related to the above rejection in the Amendment filed on November 29, 2001 in Paper No. 7 (pages 19-22) have been fully considered.

With respect to the lack of motivation for combining or modifying the cited references, Applicants mainly argued that "There is no suggestion that such MTX resistance could be counteracted by delivering an FPGS gene to neoplastic cells". Additionally, Applicants argued that "there would have been no reasonable expectation of success in addressing the problem of MTX resistance by delivering an FPGS gene neoplastic cells since lowered FPGS activity was only a hypothetical cause of the resistance". Examiner respectfully finds Applicants' arguments to be unpersuasive for the following reasons.

Firstly, with respect to the issue that lowered FPGS activity was only a hypothetical cause of the resistance of MTX, Roy et al. clearly teach that L1210 tumor cells resistant to methotrexate have a decrease in the rate of FPGS mRNA transcript formation, resulting in lower FPGS activity (page 6907, col. 2, first full paragraph), and that L1210 tumor cells resistant to edatrexate have constitutively down-regulated steady state levels of FPGS or FPGS activity compared with parental L1210 tumor cells and

that FPGS mRNA from the variant cells was significantly less effective in mediating formation of the FPGS peptide product in a manner correlating with the FPGS activity or protein (see abstract). Additionally, Pizzorno et al. (Cancer Res. 48:2149-2155, IDS) teach that the resistance to methotrexate by two human leukemia T-lymphoblast cell lines has been attributed to the substantial lower level of folate polyglutamates (their formation is mediated by FPGS activity) of these cell lines compared to parental cell lines (see abstract).

Secondly, on the basis of the teachings of Kim et al. discussed above coupled with the disclosure of Roy et al. and Pizzorno et al., it is reasonable for an ordinary skilled artisan to expect that by enhancing FPGS activity or FPGS protein via FPGS gene delivery into neoplastic cells, the methotrexate resistance will be ameliorated particularly for tumor cells that may acquire resistance to classical antifolate drugs.

Accordingly, claims 1-3 and 5-13 are rejected for the reasons set forth above.

Conclusions

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Quang Nguyen, Ph.D., whose telephone number is (703) 308-8339.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's mentor, Dave Nguyen, may be reached at (703) 305-2024, or SPE, Irem Yucel, at (703) 305-1998.

Any inquiry of a general nature or relating to the status of this application should be directed to Patent Analyst, Tracey Johnson, whose telephone number is (703) 305-2982.

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PRIMARY EXAMINER